Challenges and Solutions in high resolution 4D confocal imaging of multicellular embryonic tissues and organoids.

Following nephron development from single cell to functional unit.
Understanding of the molecular basis of collective cell migration is a modern trend in cell biology.

Box 1 | Collective cell migration in vivo

Cells that undergo collective migration are commonly found in vivo; in the pharyngeal endoderm, cranial placodes and nephric ducts, and during mammary branching morphogenesis, heart regeneration and angiogenesis.

Four well-studied in vivo models are:

- The border cell in Drosophila melanogaster (see the figure, part a). The ovary is composed of ovarioles and the egg chamber. The egg chamber contains one oocyte and several support cells, which are surrounded by epithelial follicle cells. During oogenesis, anterior polar cells (purple) recruit neighbouring cells to form the border cell cluster and start migrating. One or two leading cells extend protrusions in response to the chemotactic factor Sf1 (PGF- and VEGF-related factor 1). Spitz and Keratin, which are secreted by the oocyte, and Gurken, which is localized at the dorsal-anterior corner of the oocyte, has distinct roles during border cell migration and functions in a positive loop with Rac1 (REF. 63).

- The lateral line in zebrafish (see the figure, part b). The primordium of the zebrafish lateral line is formed by a cluster of more than 100 cells that migrates from the head to the tail of embryos, where it forms a series of rosette-like mechanosensory organs. The primordium migrates as a compact epithelial cluster with large polarized protrusions at the front. The direction of migration is determined by a self-generated gradient of Sdf1 (stromal cell-derived factor 1) (REF. 58).

- The neural crest in Xenopus laevis (see the figure, part c). Cephalic neural crests are formed in the dorsal part of the neural tube and migrate ventrally, contributing to many head structures. The cephalic neural crest undergoes an epithelial-to-mesenchymal transition (during which E-cadherin disappears) but still migrates as a cohesive cluster of cells that influence each other’s behaviour. The cluster configuration is maintained mainly by chemotaxis between neighbouring cells, instead of by cell adhesion (chemoattractant C3a, blue circles).

- Cancer invasion (see the figure, part d). During metastasis, cancer cells spread from one organ to another (for example, melanomas in the skin of the head can spread to different organs of the body). This spreading can involve collective cell migration, which is usually found in epithelial cancers, although leader cells can acquire a more mesenchymal phenotype, with cell protrusions and activation of Rac1. Often, cell clusters migrate following some physical contact with the extracellular matrix (ECM).
Developmental Cell

Real-Time Three-Dimensional Cell Segmentation in Large-Scale Microscopy Data of Developing Embryos

Graphical Abstract

Three-dimensional image data of entire membrane-labeled embryos

Fly    Fish    Mouse
Light-sheet microscopy Confocal microscopy

RACE: Realtime Accurate Cell-shape Extractor
High throughput (real-time)
High accuracy
User-friendly & open-source

Automated three-dimensional cell shape segmentation

Mutant    WT
Whole-embryo reconstruction Cell morphology comparison Joint segmentation and cell tracking Quantitative shape analysis

Highlights

- RACE framework automates 3D cell segmentation in entire fly, fish, and mouse embryos
- RACE is 55–330 times faster and 2–5 times more accurate than state-of-the-art methods
- RACE quantifies differences in cellular dynamics in wild-type and mutant embryos
- RACE + TQMM enable joint segmentation and cell tracking in entire developing embryos

Authors
Johannes Stegmaier, Fernando Armat, William C. Lemon, ..., George Teodoro, Ralf Mikut, Philipp J. Keller

Correspondence
johannes.stegmaier@kit.edu (J.S.), kellerp@janelia.hhmi.org (P.J.K.)

In Brief
Stegmaier et al. present the open-source software RACE for automated three-dimensional cell segmentation in large-scale microscopy images. RACE rapidly reconstructs cell shapes in entire developing Drosophila, zebrafish, and mouse embryos imaged with confocal or light-sheet microscopy and quantifies cell-shape dynamics and phenotypic differences in wild-type and mutant embryos.
(Vainio & Lin 2002)

(Linström 2015)
For about half century kidney embryonic culture has proven itself as a powerful tool to study the fundamental principles of nephrogenesis.

Taken from the review: “Embryonic kidney in organ culture” of Lauri Saxén and Eero Lehtonen. Differentiation 1987. 36 : 2-11

Taken from: “Mapping of the fate of cell lineages generated from cells that express the Wnt4 gene by timelapse during kidney development”. Jingdong Shan*, Tiina Jokela*, Ilya Skovorodkin and Seppo Vainio. Differentiation 2010. 79 : 57-64
Optimization of organotypic kidney culture for high resolution 4xD confocal imaging.

Fig. 1. FiDZ system. (A) In the classic Trowell system the kidney rudiment is placed on a filter supported by a grid holding the explant in the air/liquid interface to promote gas exchange. (B) In the novel FiDZ the explant is between a glass surface and a membrane. Spacer beads are used to adjust the thickness of the tissue.

Optimization of organotypic kidney culture for high resolution 4xD confocal imaging.
Application of Dissociation/Reaggregation (Organoid) method to study the basics of morphogenic program

Functional Genetic Targeting of Embryonic Kidney Progenitor Cells Ex Vivo. Juntilla et al., 2014. JASN
Application of Dissociation/Reaggregation (Organoid) method to study the basics of morphogenic program

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Application of nanocapsules for transfection of primary embryonic kidney cells

Antibody labeled by Alexa 546/{PARG/DS}_2/ Antibody labeled by Alexa 546 /DS

Mean capsules size: 300nm
Application of nanocapsules for transfection of primary embryonic kidney cells

Transfection of MM cells with Cy3-labelled Mock siRNA-containing Nanocapsules.
Optimization of Renal Embryonic Tissue Culture to follow the development of renal vasculature.
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*Ex ovo* chicken embryo culture
Optimization of Renal Embryonic Tissue Culture to follow the development of renal vasculature

Experiment (DAPI, DIC, CD31. Nephrin did not work in these samples).

Control (DAPI, Nephrin, CD31)

Mouse embryonic kidneys on CAM after 8 days of cultivation
Optimization of Renal Embryonic Tissue Culture to follow the development of renal vasculature.

Development of renal vasculature in classical mouse embryonic kidney culture (left) and in kidney culture on CAM (right). CD31 AB staining.
Task 2. Determining of the origin of the endothelial cells in kidney culture on CAM.
Chicken Cam approach

**Hypothesis:** later stages of vascularization of nephron do require the blood flow.
Application of *in vitro* perfused capillary network to study the development of renal vasculature.
STORE > Chips > 3D Cell Culture Chips DAX-1 (25/box)
Huvecs cells, Matrigel
Mouse ECs, fibrin gel
Mouse embryonic endothelial cells, fibrin gel
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